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Systemic Insecticides Reduce Feeding, Survival, and Fecundity of Adult Black Vine Weevils (Coleoptera: Curculionidae) on a Variety of Ornamental Nursery Crops

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ABSTRACT Systemic activity of the neonicotinoids clothianidin, dinotefuran, and thiamethoxam and the anthranilic diamide chlorantraniliprole was tested against adult black vine weevils, *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae), on *Astilbe*, *Euonymus*, *Heuchera*, *Rhododendron*, *Sedum*, and *Taxus*. Insecticide treatments were applied to the soilless substrate of containerized plants. Bioassays were conducted 12 or 13, 26, and 42 d after treatment (DAT) and ran for 7 d; and feeding, mortality, and weight gain or loss by weevils were evaluated. Foliage was removed from test plants and then placed in arenas with adult black vine weevils. The neonicotinoids reduced feeding and weight gain by adult black vine weevils on most plant species with residual activity 42 DAT on some plant species. At 12 DAT, mortality was caused by the three neonicotinoids on *Astilbe* and by thiamethoxam on *Sedum*; and at 26 DAT dinotefuran caused mortality on *Astilbe*. Chlorantraniliprole reduced feeding on *Taxus* at 12 DAT, with no activity detected in other bioassays. Another set of bioassays was conducted to examine survival and fecundity of adult black vine weevils during prolonged feeding on *Heuchera* and *Taxus* systemically treated with dinotefuran or thiamethoxam. Bioassay procedures were similar to those described above, except they ran continuously for 56 d. Prolonged feeding on dinotefuran and thiamethoxam treated *Heuchera* and *Taxus* resulted in high mortality of adult black vine weevils and reduced fecundity. These studies show that the systemic activity of neonicotinoids is influenced by plant species and that systemic neonicotinoids have the potential to suppress black vine weevil populations in containerized nursery crops.

KEY WORDS neonicotinoids, *Otiorhynchus sulcatus*, bioassays residual activity, chronic exposure

Black vine weevils, *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae), are serious pests in ornamental nurseries where they feed on a wide variety of plant species (Smith 1932, Masaki et al. 1984). The larvae are the most damaging stage, feeding on roots and often stunting or killing their hosts. The adult stage feeds on foliage, but the damage is usually esthetic. Black vine weevils are flightless and univoltine, and they are all females, reproducing by thelytokous parthenogenesis (Smith 1932, van Tol et al. 2004). Black vine weevils overwinter in the larval stage. In spring the larvae pupate, and then adults begin emerging by late spring to early summer, depending on climatic conditions. After emergence from the soil, the adults go through a 4–8-wk preoviposition feeding period, with the length dependent on climate and host (Stenseth 1979, Maier 1981, Nielsen and Dunlap 1981, Son and Lewis 2005).

Nurseries rely on preventive treatments of insecticides for management of black vine weevils. In container-grown crops, growers either incorporate insecticides into the potting substrate to kill the larval stage or spray foliage to kill adults during the preoviposition period before they lay eggs (Cowles 2001, Bruck and Donahue 2007). When adults are targeted three or more sprays are usually applied to cover the emergence period (Reding and Persad 2009). Pyrethroids are standard materials for control of black vine weevils, with high efficacy reported against larvae and adults (Nielsen and Montgomery 1977, Cowles 2001). However, due to environmental concerns conventional insecticides such as pyrethroids have come under increased scrutiny by the general public and regulatory agencies, which may result in loss of registrations. Furthermore, too much reliance on a single group of insecticides increases the potential for resistance to develop (Broughton and Herron 2009, Ramoutar et al. 2009); and the black vine weevil has a history of developing resistance to insecticides (Nielsen et al. 1975). Identification of additional effective insecticides should provide growers with al-

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Table 1. Insecticides and rates used in the bioassays

Active ingredient	Formulated insecticide[a]	Rate of insecticide applied (AI)	
		#1 containers	#2 containers[b]
Chlorantraniliprole	Acelepryn 1.67 SC	0.75 ml (0.150 g)	1.13 ml (0.225 g)
Clothianidin	Celero 16 WSG	0.08 g (0.013 g)	0.12 g (0.019 g)
Thiamethoxam	Flagship 25 WG[c]	0.036 g (0.009 g)	0.054 g (0.014 g)
Dinotefuran	Safari 20 SG[c]	0.22 g (0.044 g)	0.33 g (0.066 g)

[a] Acelepryn 1.67 SC (DuPont Crop Protection, Wilmington, DE); Celero 16 WSG (Arysta Lifescience North America, San Francisco, CA); Flagship 25 WG (Syngenta Crop Protection, Inc., Greensboro, NC); and Safari 20 SG (Valent U.S.A., Walnut Creek, CA).
[b] The #2 containers were used only for *Rhododendrons* in the 7-d bioassays.
[c] Only Flagship and Safari were used in the 56-d bioassays.

ternatives to current standards and slow the development of resistance to those standards.

Systemic insecticides applied to the substrate offer a potential alternative to foliar sprays for elimination of adult black vine weevils to prevent oviposition. Insecticides applied to the substrate through incorporation, drenches or drip chemigation result in less drift and worker exposure than foliar sprays. Because of the long preoviposition period, carefully timed systemic insecticides would have several weeks to impact foliar feeding adults before oviposition might occur. Reding and Persad (2009) identified several systemically active insecticides that prevented colonization of nursery plants by black vine weevil larvae and reduced feeding and survival of adults on *Sedum* plants. Further research is needed to test the systemic activity of these materials on adult black vine weevils on other host species. Variation in uptake and efficacy of systemic insecticides among plant species has been documented (Tatter et al. 1998, Poland et al. 2006). For a polyphagous pest like the black vine weevil, a systemic insecticide would have to be effective on a variety of host species to be a viable management tool.

The objectives of this study were to 1) determine the effects of systemic neonicotinoid insecticides on survival, feeding, weight gain, and fecundity of adult black vine weevils on a variety of ornamental species; and 2) evaluate the residual activity of the systemic insecticides.

Materials and Methods

Plant Species. The species of ornamental plants used were documented as good hosts for black vine weevils (Smith 1932, Nielsen and Dunlap 1981, Cowles 2001, van Tol et al. 2004, Fisher 2006, Reding 2008). In the 7-d bioassays (2008), six species were used including *Astilbe* (*Astilbe* × *arendsii* Arends 'Rheinland', Saxifragaceae), *Euonymus* [*Euonymus fortunei* (Turcz.) Hand.-Mazz. 'Coloratus', Celastraceae], *Heuchera* (*Heuchera* × *brizoides* Hort. 'Chatterbox', Saxifragaceae), *Rhododendron* (*Rhododendron catawbiense* Michx. 'Boursault', Ericaceae), *Sedum* (*Sedum spectabile* Boreau. 'Neon', Crassulaceae), and *Taxus* (*Taxus* × *media* Rehder 'Brownii', Taxaceae). In the 56-d bioassays (2009), *Heuchera* (*Heuchera* × *brizoides* 'Chatterbox') and *Taxus* (*Taxus* × *media* 'Brownii') were used. The plants were purchased bare-root and potted in #1 (≈ 3.8 liters, 15.6-cm top diameter) or #2 ([*Rhododendron* only] ≈ 7.6 liters, 21.6-cm top diameter) polyethylene containers (Premier Nursery Supplies, Hummert International, Earth City, MO) in soilless substrate (mixture of aged pine bark, 60%; peat, 15%; compost, 15%; and coarse sand, 10% by vol) at least 2 mo before being used in the bioassays. The plants were purchased during winter of the year they were used in the bioassays, and they may have been treated with systemic insecticides the season before purchase. It is not standard practice to treat plants systemically during winter dormancy. The plants were irrigated as needed from planting through the end of each experiment.

For the *Astilbe*, *Rhododendron*, and *Sedum* bioassays single leaves including petioles were used in each replication (*Astilbe* have compound leaves and replications consisted of one petiole with three leaflets). For *Euonymus*, a 5-cm section of stem with a pair of leaves was used, and for *Heuchera* one or two leaves (depending on the size), including petiole, were used. For *Taxus*, a terminal section of stem 5 cm long was used. The amount of plant tissue used was intended to supply an abundance of food for each evaluation period (3, 4, or 7 d). Immediately after removal from plants leaf petioles or stems were inserted into 2.5- by 2.5- by 3-cm pieces of water-soaked Oasis floral foam (Oasis Floral Products, Smithers-Oasis North America, Kent, OH) and placed in labeled arenas with lids, then transported to the laboratory. The arenas were 900-ml plastic containers with snap-on lids.

Insects. For the 7-d bioassays (2008), adult black vine weevils were collected from an untreated row of field-grown *Taxus* at a commercial nursery on 16 June. The weevils were kept in a screen cage and supplied with *Taxus* foliage for food until they were used in the bioassays. This cohort of adults was used for all 7-d bioassays. For the 56-d bioassays (2009), adult black vine weevils were reared from larvae infesting containerized bird's nest spruce, *Picea abies* (L.) Karst. 'Nidiformis', obtained from a commercial nursery. Adult weevils began emerging 27 April and the bioassays were started 18 May. The weevils were 2–3 wk old at the start of the bioassays and still in their preoviposition feeding period. Weevils were not starved before use in the bioassays.

Insecticides and Rates. In the 7-d bioassays (2008), the neonicotinoid insecticides clothianidin, dinotefuran, and thiamethoxam and the anthranilic diamide chlorantraniliprole were tested (see Table 1 for in-

secticide information and rates applied). In the 56-d bioassays (2009), only dinotefuran and thiamethoxam were tested. The insecticide treatments were poured onto the surface of the substrate in 120 or 180 ml of solution to #1 or #2 pots, respectively. Label rates of insecticides were based on container diameter or surface area.

Evaluation of Survival, Weight Gain, and Weevil Feeding. Weevils were weighed and survival was assessed at the start and end of each bioassay and evaluation period. For evaluation of feeding, all leaves (except *Taxus*) were scanned by a Canon imageRUNNER 1023 series (Canon U.S.A., Inc., Lake Success, NY) before being used in the bioassays. The imageRUNNER was set on scanning mode with the images downloaded directly to a desktop computer and saved as TIFF files at 29.53 pixels per cm (75 dpi). After scanning, the leaf petioles and stems were reinserted into Oasis floral foam and placed back in the assay arenas. At the end of an evaluation period, the leaves were rescanned.

Photoshop version 8.0 (Adobe Systems, Inc., San Jose, CA) was used to measure the surface area of the leaf images by determining the number of pixels for the remaining leaf tissue. Adult black vine weevils feed along the margins of leaves creating areas of injury with all leaf material removed, thus only the remaining leaf tissue is measured by the software. Pixels of leaf tissue were converted to square centimeters. Feeding was measured as the area of leaf (square centimeters) eaten by weevils (difference in pixels between the starting and final leaf images), except on *Taxus* where feeding notches were quantified because the stems did not scan reliably (Reding and Persad 2009). In the *Taxus* assessments, if the end of a needle was removed that was counted as one feeding-notch.

A control bioassay without weevils was run on each plant species to test measurements of leaf area. There was an increase in size, presumably due to loss of turgor (leaf tissue would spread more when pressed in the scanner), for all species. Based on the control assay, postassay measurements of leaf area were reduced by 16.7, 6.8, 11.4, 1, and 4.3 for *Astilbe*, *Euonymus*, *Heuchera*, *Rhododendron*, and *Sedum*, respectively.

7-d Bioassays (2008). In the 7-d bioassays, systemic insecticides were tested on six species of ornamental nursery crops to evaluate affects on survival, feeding, and weight gain of adult black vine weevils. The experiments were completely randomized designs with seven replications (arenas) per treatment; and there were seven plants of each species per treatment (one plant per replication). These bioassays ran for 7 d and were kept at ambient temperature (21–25°C) on a bench in the laboratory. To evaluate residual activity of insecticides and the potential for a single treatment to provide season-long control, bioassays were run at three posttreatment timings (12 or 13, 26, and 42 d). The plants were treated on 11 June 2008 and the first set of bioassays began 12 or 13 d after that (23 or 24 June, *Taxus*, *Heuchera*, *Astilbe* or *Sedum*, *Euonymus*, *Rhododendron*, respectively). The second and third sets of bioassays began 26 and 42 d after treating (all species), respectively. The same plants were sampled for each posttreatment timing. Hereafter, the three bioassay timings (12 or 13, 26, and 42 d) will be referred to as bioassay 1, bioassay 2, and bioassay 3. At the start of each assay, one preweighed (milligrams) adult black vine weevil was placed in each arena, and then the lids were applied.

56-d Bioassays (2009). The 56-d bioassays were designed to determine whether prolonged feeding on foliage treated systemically with dinotefuran or thiamethoxam influences survival or fecundity of adult black vine weevils. The experiments were repeated measures designs (the same weevils and plants were used throughout each bioassay) with 10 single-plant replications per treatment. There was one bioassay arena per plant with two adult black vine weevils per arena. The bioassay replications corresponded with a specific plant so that weevils in each replication were presented only with foliage from the corresponding plant. For example, the weevils in replication one of the *Taxus* dinotefuran treatment were presented with foliage from plant 1 of the *Taxus* dinotefuran treatment for the entire assay. Insecticide treatments were applied 12 d before the bioassays began. The bioassays were kept in a temperature controlled room at 24°C and a photoperiod of 16:8 (L:D) h and evaluated at 3- to 4-d intervals. During each evaluation, amount of feeding, number of eggs laid, and survival were determined. After each evaluation, weevils were placed in clean arenas and supplied with fresh foliage from the treatment plants and fresh Oasis floral foam.

Data Analysis. Data for each plant species were analyzed separately. Data were analyzed by analysis of variance (ANOVA) for a completely randomized design or repeated measures design for the 7- or 56-d bioassays, respectively; and following a significant ANOVA Dunnett's test was used to compare insecticide treatments ($\alpha = 0.05$) with the control (untreated plants) (Zar 1999, Analytical Software 2003). Data with heterogeneous variances were transformed [$\log (X + 1)$] before analysis (Zar 1999). Feeding was analyzed at the end of each evaluation period as the mean leaf area (square centimeters) eaten (*Astilbe*, *Euonymus*, *Heuchera*, *Rhododendron*, and *Sedum*) or mean number of feeding notches (*Taxus*). Survival was analyzed as the mean number of live weevils at the end of each evaluation period. In the 56-d *Taxus* bioassay, one weevil went missing in the control treatment after the first evaluation period. Afterward, calculations of survival in that treatment were based on 19 weevils instead of 20. In the 7-d bioassays, feeding, survival, and change in weight of weevils were analyzed within each posttreatment timing. Replications with dead weevils were not included in the weight analysis.

In the 56-d bioassays, for each evaluation period the amount of feeding in a replication was averaged by the number of live weevils at the start of that evaluation period. This allowed us to include in the analysis replications where weevils died during an evaluation period and include replications with one or two surviving weevils. After both weevils in a replication died, that

Table 2. Results of bioassay 1 (12 or 13 DAT), amount of feeding (mean ± SD amount of leaf tissue removed [square centimeters] or number of notches [*Taxus*]), survival (total number of live weevils per treatment [percentage of survivorship]), and change in weight (mean ± SD milligram increase or decrease) of adult black vine weevils during 7-d bioassays

Variable	Treatment	Plant species[a]					
		Taxus	*Heuchera*	*Astilbe*	*Sedum*	*Euonymus*	*Rhododendron*
Feeding[b]	Control	30.6 ± 4.1	12.9 ± 1.3	11.1 ± 3.3	3.0 ± 1.6	5.6 ± 0.9	2.4 ± 1.7
	Dinotefuran	12.7 ± 3.5*	4.1 ± 0.9*	5.6 ± 1.5*	0.4 ± 0.4*	1.5 ± 0.5*	0.6 ± 0.3*
	Clothianidin	11.3 ± 3.6*	7.4 ± 3.1*	9.3 ± 3.0	1.0 ± 0.6*	2.9 ± 0.9*	3.8 ± 0.9
	Chlorantraniliprole	24.7 ± 4.5*	13.2 ± 2.8	12.7 ± 2.6	3.3 ± 0.7	5.3 ± 0.8	3.3 ± 2.5
	Thiamethoxam	15.4 ± 4.7*	5.7 ± 3.1*	7.5 ± 3.2	1.1 ± 0.3*	2.0 ± 0.5*	0.5 ± 0.3*
	F	28.8	20.5	7.09	19.6[c]	48.7	10.0[c]
	df	4, 30	4, 30	4, 30	4, 30	4, 30	4, 30
	P	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	<0.0001
Survival	Control	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	5 ± 0.5 (71.4)
	Dinotefuran	6 ± 0.4 (85.7)	6 ± 0.4 (85.7)	1 ± 0.4 (14.3)*	7 ± 0.0 (100)	5 ± 0.5 (71.4)	6 ± 0.4 (85.7)
	Clothianidin	7 ± 0.0 (100)	5 ± 0.5 (71.4)	2 ± 0.5 (28.6)*	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)
	Chlorantraniliprole	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	6 ± 0.4 (85.7)	7 ± 0.0 (100)	6 ± 0.4 (85.7)
	Thiamethoxam	7 ± 0.0 (100)	5 ± 0.5 (71.4)	3 ± 0.5 (42.9)*	2 ± 0.5 (28.6)*	5 ± 0.5 (71.4)	7 ± 0.0 (100)
	F	1.0	1.15	8.57	8.81	1.8	0.95
	df	4, 30	4, 30	4, 30	4, 30	4, 30	4, 30
	P	0.423	0.351	0.0001	0.0001	0.155	0.447
Change in wt	Control	10.0 ± 5.5	11.1 ± 3.8	7.6 ± 2.8	−5.1 ± 5.4	5.7 ± 4.1	3.6 ± 3.2
	Dinotefuran	−6.0 ± 4.5*	0.8 ± 4.7*	na[d]	−15.1 ± 2.1*	−15.2 ± 4.4*	−11.3 ± 7.6*
	Clothianidin	−10.0 ± 7.2*	2.6 ± 6.3*	na	−11.3 ± 7.9	−3.1 ± 4.8*	2.4 ± 3.3
	Chlorantraniliprole	7.0 ± 3.6	5.7 ± 4.8	9.3 ± 3.0	−4.7 ± 4.8	7.7 ± 3.8	5.7 ± 7.2
	Thiamethoxam	3.6 ± 7.4	0.6 ± 7.2*	na	na	−6.2 ± 7.4*	−8.6 ± 5.0*
	F	14.5	4.38	1.2	5.71	21.0	11.8
	df	4, 29	4, 25	1, 12	3, 23	4, 26	4, 26
	P	<0.0001	0.008	0.296	0.025	<0.0001	<0.0001

[a] Means followed by an asterisk (*) are significantly different than the control ($\alpha = 0.05$; Dunnett's test).
[b] Final leaf area was corrected for increase in leaf size due to loss of turgor; thus, the leaves spread when pressed in the scanner.
[c] Analysis of transformed [log(X + 1)] data.
[d] Not applicable.

replication was excluded from the feeding analysis in subsequent evaluation periods. Because there was no data on feeding for excluded replications, analyses ended when the number of active replications within at least one treatment dropped to less than five. In the survival analysis, all replications were included through the ends of the bioassays. Fecundity was analyzed as mean cumulative eggs laid during the entire bioassay.

Results

7-d Bioassays. In bioassay 1 (12 or 13 d after treatment [DAT]), feeding was suppressed by dinotefuran on all species; by thiamethoxam on all species except *Astilbe;* by clothianidin on *Euonymus*, *Heuchera*, *Sedum*, and *Taxus;* and by chlorantraniliprole on *Taxus* (Table 2). Significant mortality was caused by dinotefuran, clothianidin, and thiamethoxam on *Astilbe;* and by thiamethoxam on *Sedum* (Table 2). There was no significant mortality on other species. Because of high mortality, the neonicotinoids were excluded from the weight analysis on *Astilbe*, and thiamethoxam was excluded from *Sedum.* Weight gain was suppressed by dinotefuran on all other species; by clothianidin on *Euonymus*, *Heuchera*, and *Taxus;* and by thiamethoxam on *Euonymus*, *Heuchera*, and *Rhododendron* (Table 2).

In bioassay 2 (26 DAT), feeding was suppressed by dinotefuran on all species except *Astilbe* and *Heuchera;* by clothianidin on *Euonymus*, *Sedum*, and *Taxus;* and by thiamethoxam on *Rhododendron*, *Sedum*, and *Taxus* (Table 3). The only significant mortality was caused by dinotefuran on *Astilbe* (Table 3). Weight gain was suppressed by dinotefuran on all species except *Astilbe* and *Sedum;* and by clothianidin and thiamethoxam on *Heuchera* and *Taxus* (Table 3).

In bioassay 3 (42 DAT), feeding was suppressed by dinotefuran on *Taxus*, *Heuchera*, and *Euonymus;* by clothianidin on *Taxus*, *Heuchera*, and *Sedum;* and by thiamethoxam on *Taxus* and *Heuchera* (Table 4). There was no significant mortality in bioassay 3 (Table 4). Weight gain was suppressed by dinotefuran on *Taxus*, *Euonymus*, and *Rhododendron* and by clothianidin on *Taxus* and *Heuchera* (Table 4).

56-d Bioassays. At 56 d, survival in the *Taxus* assay was 78.9, 10, and 0% in the control, thiamethoxam, and dinotefuran treatments, respectively (Fig. 1). All weevils in the dinotefuran treatment were dead after 49 d. In the *Taxus* assay, significant differences in survival occurred for the first time at 28 d with 84.2, 35, and 30% survival in the control, thiamethoxam, and dinotefuran treatments, respectively ($F = 6.35$; df = 2, 18; $P = 0.008$) (Fig. 1); and from that time on, survival was always significantly higher in the control than the insecticide treatments ($F > 6.35$; df = 2, 18; $P < 0.01$) (Fig. 1). In the *Heuchera* assay, survival at 56 d was 30, 0, and 0% in the control, thiamethoxam, and dinotefuran treatments, respectively (Fig. 2). No weevils survived for >28 d in the thiamethoxam or dinotefuran treatments in the *Heuchera* assay, whereas at that time survival in the controls was 50% (Fig. 2). The *Heuchera* bioassay was continued beyond 28 d to evaluate fe-

Table 3. Results of bioassay 2 (26 DAT), amt of feeding [mean ± SD amt of leaf tissue removed (cm^2) or no. of notches (*Taxus*)], survival [total no. of live weevils per treatment] (percent survivorship), and change in wt [mean ± SD mg increase or decrease] of adult black vine weevils during 7-d bioassays

Variable	Treatment	Plant species[a]					
		Taxus	*Heuchera*	*Astilbe*	*Sedum*	*Euonymus*	*Rhododendron*
Feeding[b]	Control	32.4 ± 9.5	8.4 ± 3.0	6.9 ± 2.2	0.7 ± 0.6	2.2 ± 0.8	1.0 ± 0.8
	Dinotefuran	11.6 ± 3.1*	4.9 ± 1.3	5.3 ± 0.8	−0.5 ± 0.7*	0.4 ± 0.8*	−0.2 ± 0.2*
	Clothianidin	8.7 ± 3.8*	5.5 ± 1.0	6.2 ± 1.9	−0.5 ± 0.8*	0.8 ± 1.3*	1.2 ± 1.4
	Chlorantraniliprole	26.7 ± 7.4	7.8 ± 3.4	11.7 ± 1.7*	0.4 ± 1.1	2.6 ± 0.7	1.6 ± 1.0
	Thiamethoxam	13.6 ± 6.8*	5.0 ± 1.5	6.4 ± 0.7	−0.4 ± 0.7*	1.1 ± 0.5	−0.1 ± 0.4*
	F	17.5	2.66[c]	18.1	3.66	7.85	6.17[c]
	df	4, 30	4, 30	4, 30	4, 30	4, 30	4, 30
	P	<0.0001	0.052	<0.0001	0.015	0.0002	0.001
Survival	Control	7 ± 0.0 (100)	6 ± 0.4 (85.7)	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)
	Dinotefuran	7 ± 0.0 (100)	7 ± 0.0 (100)	4 ± 0.5 (57.1)*	7 ± 0.0 (100)	6 ± 0.4 (85.7)	6 ± 0.4 (85.7)
	Clothianidin	7 ± 0.0 (100)	6 ± 0.4 (85.7)	6 ± 0.4 (85.7)	6 ± 0.4 (85.7)	7 ± 0.0 (100)	6 ± 0.4 (85.7)
	Chlorantraniliprole	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	6 ± 0.4 (85.7)	7 ± 0.0 (100)	7 ± 0.0 (100)
	Thiamethoxam	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	6 ± 0.4 (85.7)	7 ± 0.0 (100)	6 ± 0.4 (85.7)
	F	na	0.75	2.83	0.50	1.00	0.50
	df	4, 30	4, 30	4, 30	4, 30	4, 30	4, 30
	P	na	0.566	0.042	0.736	0.423	0.736
Change in wt	Control	−1.1 ± 1.7	4.7 ± 4.1	−2.1 ± 4.5	−4.6 ± 3.8	−0.4 ± 2.1	−2.9 ± 9.5
	Dinotefuran	−8.6 ± 4.0*	−8.4 ± 6.6*	−1.0 ± 2.2	−9.4 ± 4.8	−6.7 ± 3.6*	−11.5 ± 3.6*
	Clothianidin	−11.3 ± 4.5*	−5.8 ± 4.9*	−3.8 ± 2.2	−7.0 ± 5.5	−0.7 ± 1.4	0.2 ± 6.0
	Chlorantraniliprole	−1.6 ± 2.0	−0.7 ± 3.7	−2.4 ± 4.9	−4.7 ± 2.4	3.1 ± 4.1	0.4 ± 2.1
	Thiamethoxam	−6.6 ± 4.1*	−5.1 ± 2.7*	0.1 ± 1.3	−6.5 ± 3.8	−2.9 ± 3.2	−6.8 ± 3.9
	F	11.5	8.02	0.90	1.53	9.35	4.80
	df	4, 30	4, 28	4, 26	4, 27	4, 29	4, 27
	P	<0.0001	0.0002	0.477	0.223	0.0001	0.005

[a] Means followed by an asterisk (*) are significantly different than the control ($\alpha = 0.05$; Dunnett's test).
[b] Final leaf area was corrected for increase in leaf size due to loss of turgor; thus, the leaves spread when pressed in the scanner.
[c] Analysis of transformed [log(X + 1)] data.

cundity of adult black vine weevils on the control plants. There were significant differences in survival for the first time at 17 d with higher survival in the control than both insecticide treatments ($F = 4.90$; df = 2, 18; $P = 0.02$) (Fig. 2); and from that point on, survival in the control treatment was always significantly higher than both insecticide treatments ($F > 4.90$; df = 2, 18; $P < 0.01$) (Fig. 2).

On *Taxus*, there were significant differences in feeding for each evaluation date through 31 d (Table 5); at that time, there was too much mortality for subsequent feeding analyses. There was more feeding on

Table 4. Results of bioassay 3 (42 DAT), amount of feeding (mean ± SD amount of leaf tissue removed [square centimeters] or number of notches [*Taxus*]), survival (total number of live weevils per treatment) [percentage of survivorship]), and change in weight (mean ± SD milligram increase or decrease) of adult black vine weevils during 7-d bioassays

Variable	Treatment	Plant species[a]					
		Taxus	*Heuchera*	*Astilbe*	*Sedum*	*Euonymus*	*Rhododendron*
Feeding[b]	Control	24.1 ± 10.4	7.4 ± 2.3	6.9 ± 2.0	1.8 ± 0.5	3.2 ± 0.7	1.6 ± 1.3
	Dinotefuran	13.1 ± 4.7*	5.1 ± 1.4*	6.7 ± 1.3	0.9 ± 0.5	1.8 ± 0.6*	0.7 ± 0.5
	Clothianidin	7.6 ± 2.9*	4.3 ± 1.3*	6.9 ± 1.7	0.4 ± 0.4*	2.1 ± 1.2	1.1 ± 0.9
	Chlorantraniliprole	23.9 ± 6.9	8.5 ± 1.8	8.6 ± 1.6	1.7 ± 1.2	3.1 ± 1.0	1.0 ± 1.0
	Thiamethoxam	11.0 ± 6.6*	4.4 ± 0.6*	6.3 ± 0.9	1.0 ± 0.6	2.6 ± 0.4	0.3 ± 0.4
	F	8.87	10.2	2.27	10.5[c]	3.78	2.16
	df	4, 30	4, 30	4, 30	4, 30	4, 30	4, 30
	P	0.0001	<0.0001	0.086	<0.0001	0.013	0.098
Survival	Control	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)
	Dinotefuran	7 ± 0.0 (100)	6 ± 0.4 (85.7)	7 ± 0.0 (100)	5 ± 0.5 (71.4)	7 ± 0.0 (100)	7 ± 0.0 (100)
	Clothianidin	7 ± 0.0 (100)	7 ± 0.0 (100)	6 ± 0.4 (85.7)	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)
	Chlorantraniliprole	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)
	Thiamethoxam	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)	7 ± 0.0 (100)
	F	na[d]	1.0	1.0	2.40	na	na
	df	4, 30	4, 30	4, 30	4, 30	4, 30	4, 30
	P	na	0.423	0.423	0.072	na	na
Change in wt	Control	13.0 ± 4.5	14.0 ± 4.1	10.4 ± 4.0	7.7 ± 2.4	12.3 ± 2.9	9.0 ± 6.1
	Dinotefuran	3.7 ± 4.2*	10.2 ± 4.2	10.7 ± 3.5	2.6 ± 3.0	5.3 ± 4.0*	−1.6 ± 5.3*
	Clothianidin	2.9 ± 4.9*	7.9 ± 3.8*	11.7 ± 5.2	6.4 ± 4.4	9.7 ± 6.1	6.7 ± 6.1
	Chlorantraniliprole	11.3 ± 3.8	13.1 ± 5.0	13.4 ± 4.2	7.9 ± 3.2	11.4 ± 4.8	7.1 ± 4.1
	Thiamethoxam	7.6 ± 4.3	8.4 ± 4.1	10.7 ± 2.8	6.1 ± 4.5	12.4 ± 3.4	6.6 ± 3.6
	F	7.35	2.93	0.68	1.89	3.16	4.45
	df	4, 30	4, 29	4, 29	4, 28	4, 30	4, 30
	P	0.0003	0.038	0.611	0.139	0.028	0.006

[a] Means followed by an asterisk (*) are significantly different than the control ($\alpha = 0.05$; Dunnett's test).
[b] Final leaf area was corrected for increase in leaf size due to loss of turgor; thus, the leaves spread when pressed in the scanner.
[c] Analysis of transformed [log(X + 1)] data.
[d] Not applicable.

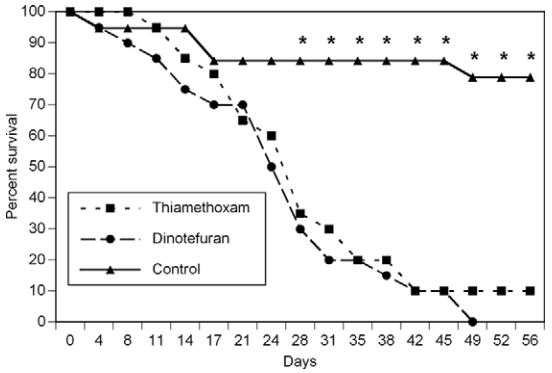

Fig. 1. Survival of adult black vine weevils over time during prolonged feeding on *Taxus* systemically treated with substrate drenches of thiamethoxam or dinotefuran. The asterisks (*) designate significant differences in survival between both insecticide treatments and the control ($P < 0.05$; repeated measures ANOVA).

the control *Taxus* than thiamethoxam and dinotefuran treated plants on all but one evaluation date; at 8 d, the thiamethoxam treatment was similar to the control (Table 5). On *Heuchera*, there were significant differences in feeding for each evaluation date through 14 d, with more feeding in the control treatment than both insecticide treatments (Table 6); except at 11 d where there was no difference between the control and thiamethoxam treatments (Table 6). At 17 and 21 d, there were no differences among treatments (Table 6); and after 21 d, there was too much mortality to continue the feeding analysis.

Very few eggs were laid in the insecticide treatments on *Taxus* or *Heuchera*, with significantly more eggs laid in the control treatments than either insecticide treatment on both hosts ($F = 42.34$; df = 2, 18; $P < 0.0001$; and $F = 11.82$; df = 2, 18; $P = 0.0005$, respectively). During the 56-d assay period, adult black vine weevils laid 118.9 ± 84.6, 0.5 ± 1.0, and 4.0 ± 6.2 eggs (mean ± SD) in the control, dinotefuran, and thiamethoxam treatments, respectively, on *Taxus;* and on *Heuchera* 56.9 ± 79.1, 0.2 ± 0.4, and 0.2 ± 0.4 eggs in the control, dinotefuran, and thiamethoxam treatments, respectively.

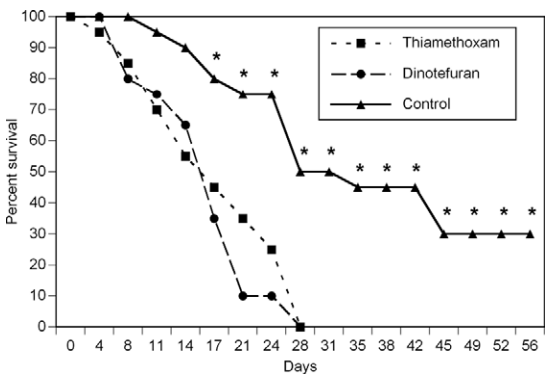

Fig. 2. Survival of adult black vine weevils over time during prolonged feeding on *Heuchera* systemically treated with substrate drenches of thiamethoxam or dinotefuran. The asterisks (*) designate significant differences in survival between both insecticide treatments and the control ($P < 0.05$; repeated measures ANOVA).

Discussion

In the current study, the systemic activity of the insecticides was variable among plant species. Previous studies also have shown variability in the systemic activity of neonicotinoid insecticides among plant species (Tatter et al. 1998, Poland et al. 2006). Poland et al. (2006) found that efficacy of imidacloprid and thiacloprid against the Asian longhorned beetle, *Anoplophora glabripennis* Motchulsky, varied among tree species. Tatter et al. (1998) found that uptake of imidacloprid varied among tree species whether applied to the soil or injected into the trees. Variability in activity of neonicotinoid insecticides among plant species may be related to differential uptake and movement within the plants. Clothianidin, dinotefuran, and thiamethoxam differ in water solubility and liphophilicity, which would influence their movement within plants and uptake by the roots, respectively (Jeschke and Nauen 2008).

In the 7-d bioassays, the insecticides were most consistently active on *Taxus* and *Heuchera* with all three neonicotinoids suppressing either feeding or weight gain through 42 d after treatment. In general, low activity was detected on *Rhododendron* through the three bioassay timings. The *Rhododendron* plants were relatively large and were the only plants potted in #2 containers. The lack of insecticide activity may have resulted from insufficient doses of insecticides. The #2 containers were approximately twice the volume of the #1 containers; however, because the labeled rates of the insecticides are based on surface area or pot diameter, instead of volume, the amount of insecticide applied was increased by only one third. Prolonged exposure to dinotefuran and thiamethoxam produced similar results on *Heuchera* and *Taxus.* Both insecticides suppressed feeding, caused high mortality, and reduced fecundity. However, on *Heuchera* significant mortality occurred 11 d earlier than on *Taxus;* and on *Heuchera* both insecticides caused 100% mortality within 28 d. In contrast, 100% mortality occurred at 49 d in the dinotefuran treatment on *Taxus*, whereas mortality in the thiamethoxam treatment was only 90% by 56 d.

Chlorantraniliprole is labeled as a systemic insecticide, however, it exhibited very little systemic activity in the current study. These results are similar to a previous study where chlorantraniliprole applied to the substrate did not reduce feeding by adult black vine weevils on the foliage of containerized *Sedum* plants (Reding and Persad 2009). In the same study, chlorantraniliprole suppressed larval populations (Reding and Persad 2009). It is possible that chlorantraniliprole has low activity against adult black vine weevils. Alternatively, chlorantraniliprole may have poor systemic activity when applied to the roots of

Table 5. Statistics from analysis of feeding by adult black vine weevils on *Taxus* treated systemically with thiamethoxam or dinotefuran; and the mean number of feeding notches for each evaluation period

Days[a]	Statistics			Mean notches ± SD[b]		
	F	df	*P*	Control	Thiamethoxam	Dinotefuran
4	10.29	2, 18	0.001	11.0 ± 2.4	7.2 ± 2.0*	7.4 ± 1.9*
8	14.90	2, 18	0.0002[c]	17.8 ± 4.8	14.9 ± 4.7	7.3 ± 2.3*
11	31.90	2, 18	<0.0001	16.3 ± 3.1	7.7 ± 2.6*	7.5 ± 3.0*
14	7.78	2, 18	0.004	16.1 ± 5.8	8.9 ± 4.8*	7.3 ± 3.7*
17	6.71	2, 17	0.007	15.4 ± 6.1	9.6 ± 5.1*	6.4 ± 2.8*
21	16.58	2, 17	0.0001	20.3 ± 5.6	11.5 ± 6.4*	8.3 ± 4.1*
24	7.23	2, 15	0.006	14.4 ± 6.7	6.7 ± 3.7*	6.4 ± 4.2*
28	6.29	2, 14	0.011	14.2 ± 7.3	5.0 ± 5.4*	3.9 ± 2.9*
31	7.16	2, 9	0.014	15.2 ± 4.2	7.4 ± 5.1*	4.9 ± 2.4*

[a] Days after the start of the bioassay. Analysis of feeding ended when at least one treatment had <5 replications with live weevils.
[b] Means followed by an asterisk (*) are significantly different than the control as determined by repeated measures ANOVA ($P < 0.05$) and Dunnett's test ($\alpha = 0.05$).
[c] Data are log(X + 1) transformed for analysis.

plants because it has a very low water solubility (1.0 mg/liter) compared with the tested neonicotinoids (DuPont 2010), which would cause poor mobility through the xylem and inhibit transport to the foliage where adult black vine weevils feed (Jeschke and Nauen 2008).

In the 56-d bioassays, feeding was deterred by the insecticide treatments during most evaluation periods analyzed. However, continuous exposure to insecticide-treated foliage did not lead to a complete cessation of feeding. It took at least 17 or 28 d of continuous exposure to treated *Heuchera* or *Taxus* foliage, respectively, to cause significant mortality. The weevils fed for at least 2 wk on untreated *Taxus* before being used in these bioassays, which may have prolonged their survival on the insecticide treated foliage. Mortality might have been caused by a combination of insecticide toxicity and weevils being weakened by reduced food intake. In a nursery, adult weevils weakened by prolonged feeding on foliage treated with systemic insecticides might be more susceptible to predation or pathogens (Amiri et al. 1999, Shah et al. 2007). Weight loss or low fecundity due to low palatability of treated hosts may lead to dispersal by weevils in search of more suitable hosts (Maier 1978, Moorhouse et al. 1992, Cowles 2004b). Dispersal would increase exposure to predation, which could increase mortality (Maier 1978). However, if dispersing weevils locate untreated hosts their probability of survival and oviposition should increase (Cowles 2004a). To prevent this, growers should treat all susceptible plant species.

Dinotefuran and thiamethoxam treatments reduced the numbers of eggs laid by adult black vine weevils on *Heuchera* and *Taxus.* This reduction was primarily the result of high mortality of weevils in those treatments. In the controls for both hosts, >90% of the eggs were laid after 31 d. By that time most weevils were dead in the insecticide treatments, whereas those surviving beyond that time rarely laid eggs. The lack of oviposition by surviving weevils may have resulted, in part, from reduced feeding. Reducing food intake by black vine weevils has been associated with reduced egg production (Shanks and Doss 1986, Doss and Shanks 1988). Furthermore, Shanks and Doss (1986) found that reducing food intake increased the length of the preoviposition period of black vine weevils, which also can decrease fecundity (Maier 1981, Fisher 2006).

Management of black vine weevils in nurseries relies primarily on conventional insecticides, especially pyrethroids (Cowles 2001, Reding and Persad 2009). However, development of resistance is possible and registration review of pyrethroids could lead to loss of registrations especially on specialty crops such as ornamental plants (Nielsen et al. 1975, USEPA 2010). Neonicotinoids are relatively low risk for nontarget organisms, have been effective in resistance manage-

Table 6. Statistics from analysis of feeding by adult black vine weevils on *Heuchera* treated systemically with thiamethoxam or dinotefuran; and the mean leaf area (square centimeters) eaten

Days[a]	Statistics			Mean ± SD leaf area[b]		
	F	df	*P*	Control	Thiamethoxam	Dinotefuran
4	11.78	2, 18	0.0005[c]	2.5 ± 1.2	0.9 ± 1.0*	0.5 ± 0.4*
8	10.58	2, 18	0.0009	2.3 ± 1.0	1.0 ± 1.2*	0.4 ± 0.5*
11	4.18	2, 17	0.033	1.2 ± 0.7	0.8 ± 0.7	0.5 ± 0.6*
14	7.79	2, 16	0.004	1.1 ± 0.5	0.5 ± 0.5*	0.4 ± 0.2*
17	0.03	2, 13	0.968	0.6 ± 0.8	0.6 ± 0.5	0.6 ± 0.4
21	0.06	2, 10	0.939	0.6 ± 0.4	0.5 ± 0.6	0.5 ± 0.6

[a] Days after the start of the bioassay. Analysis of feeding ended when at least one treatment had <5 replications with live weevils.
[b] Means followed by an asterisk (*) are significantly different than the control as determined by repeated measures ANOVA ($P < 0.05$) and Dunnett's test ($\alpha = 0.05$).
[c] Data are log(X + 1) transformed for analysis.

ment programs, and show promise as management tools for black vine weevils (Jeschke and Nauen 2008, Reding and Persad 2009). In the current study, 28–56-d exposure to dinotefuran and thiamethoxam treated foliage caused 90–100% mortality of adults and reduced the numbers of eggs laid by 97–99%. Although residual activity of clothianidin, dinotefuran, and thiamethoxam lasted at least 42 d on some but not all plant species tested. In a management program for black vine weevils, these materials could be applied to the substrate of containerized plants when adults begin emerging. At that time, the treatments should cause significant mortality of the adult weevils during the preoviposition period; or at least prolong the preoviposition period and reduce fecundity by suppressing feeding (Maier 1981, Shanks and Doss 1986, Doss and Shanks 1988, Fisher 2006). In addition, clothianidin and dinotefuran have been shown to prevent infestations by black vine weevil larvae (Reding and Persad 2009). The dual activity of these materials against adults and larvae should provide effective control of black vine weevils with a single treatment. Because there were differences in systemic activity of the neonicotinoids among plant species, further research is needed on other black vine weevil hosts. In addition, further testing is needed to determine activity against the larval stage, and evaluate residual activity on both stages. Materials that provide season-long control with a single treatment would be the most acceptable to growers.

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